WE CLAIM:

1. A process for the detection of a specific nucleic acid sequence, comprising the steps of:

(a) Providing a single reaction medium containing reagents comprising

(i) a first oligonucleotide primer,

(ii) a second oligonucleotide primer comprising an antisense sequence of a promoter,

(iii) a DNA-directed RNA polymerase that recognizes said promoter,

(iv) an RNA-directed DNA polymerase,

(v) a DNA-directed DNA polymerase,

(vi) a ribonuclease that hydrolyses RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA,

(b) Providing in said reaction medium RNA comprising an RNA first-template which comprises said specific nucleic acid sequence or a sequence complementary to said specific nucleic acid sequence, under conditions such that a cycle ensues wherein

(i) said first oligonucleotide primer hybridizes to said RNA first template,

(ii) said RNA-directed DNA polymerase uses said RNA first template to synthesize a DNA second template by extension of said first oligonucleotide primer and thereby forms an RNA-DNA hybrid intermediate,

(iii) said ribonuclease hydrolyses RNA which comprises said RNA-DNA hybrid intermediate,

(iv) said second oligonucleotide primer hybridizes to said DNA second template,

(v) said DNA-directed DNA polymerase uses said second oligonucleotide primer as template to synthesize said promoter by extension of said DNA second template; and

(vi) said DNA-directed RNA polymerase recognizes said promoter and transcribes said DNA second template,

thereby providing copies of said RNA first template;
and thereafter

(c) Maintaining said conditions for a time sufficient to achieve a desired amplification of said specific nucleic acid sequence, followed by the addition of;

(i) at least one probe sequence complementary to said RNA first template labeled with an electrochemiluminescent species,

(ii) at least one second capture probe sequence complementary to said RNA first template labeled with a binding species,

(iii) a bead coated with a complementary binding species to said second probe sequence; and thereafter

(d) Providing conditions of temperature and buffer to allow the hybridization of the probes to the said RNA first template and the binding of said binding species on said second capture probe with the complementary binding species on said bead to from a bead bound complex; and then

(e) Detecting said bead bound complex using said electrochemiluminescent species.

2. A process according to claim 1, wherein said RNA first template comprises siad specific nucleic acid sequence and wherein step (B) comprises providing single-stranded RNA in said reaction medium such that

(i) said first oligonucleotide primer hybridizes to said single-stranded RNA,

(ii) said RNA-directed DNA polymerase uses said single-stranded RNA as a template to synthesize a DNA second template by extension of said first oligonucleotide primer and thereby forms an RNA-DNA hybrid,

(iii) said ribonuclease hydrolyses RNA which comprises said RNA-DNA hybrid,

(iv) said second oligonucleotide primer hybridizes to said DNA second template,

(v) said DNA-directed DNA polymerase uses said second oligonucleotide primer as template to synthesize said promoter by extension of said DNA second template; and

(vi) said DNA-directed RNA polymerase recognizes said promoter and transcribes said DNA second template, thereby providing copies of said RNA first template.

3. A process according to claim 1, wherein said RNA first template comprises a sequence complementary to said specific nucleic acid sequence and wherein step (B) comprises providing single-stranded RNA in said reaction medium such that

(i) said second oligonucleotide primer hybridizes to said single-stranded RNA,

(ii) said RNA-directed DNA polymerase uses said RNA as a template to synthesize a complementary DNA by extension of said second oligonucleotide primer and thereby forms an RNA-DNA hybrid,

(iii) said ribonuclease hydrolyses RNA which comprises said RNA-DNA hybrid,

(iv) said first oligonucleotide primer hybridizes to said complementary DNA,

(v) said DNA-directed DNA polymerase uses said complementary DNA as template to synthesize said DNA second template and said promoter by extension of said first oligonucleotide primer; and

(vi) said DNA-directed RNA polymerase recognizes said promoter and transcribes said DNA second template, thereby providing copies of said RNA first template.

4. A process according to claim 1, wherein step (B) comprises adding to said reaction medium single-stranded DNA which comprises an antisense sequence of said promoter, such that

(i) said first oligonucleotide primer hybridizes to said single-stranded DNA,

(ii) said DNA-directed DNA polymerase uses said single-stranded RNA as a template to synthesize said DNA second template and said promoter by extension of said first oligonucleotide primer; and

(iii) said DNA-directed RNA polymerase recognizes said promoter and transcribes said DNA second template, thereby providing copies of said RNA first template.

5. A process according to claim 4, wherein step (B) comprises adding to said reaction medium and RNA-DNA hybrid comprising said single-stranded DNA, such that said ribonuclease hydrolyzes RNA which comprises said RNA-DNA hybrid.

6. A process according to claim 1, wherein step (B) comprises adding to said reaction medium single-stranded DNA which comprises said DNA second template, such that

(i) said second oligonucleotide primer hybridizes to said single-stranded DNA,

(ii) said DNA-directed DNA polymerase uses said second oligonucleotide primer as template to synthesize said promoter by extension of said DNA second template;

and

(iii) said DNA-directed RNA polymerase recognizes said promoter and transcribes said DNA second template, thereby providing copies of said RNA first template.

7. A process according to claim 6, wherein step (B) comprises adding to said reaction medium and RNA-DNA hybrid comprising said single-stranded DNA, such that said ribonuclease hydrolyzes RNA which comprises said RNA-DNA hybrid.

8. A process according to claim 2, wherein step (B) comprises adding to said reaction medium a DNA comprising said promoter, such that said DNA-directed RNA polymerase transcribes said DNA, thereby synthesizing said single-stranded RNA.

9. A process according to claim 3, wherein step (B) comprises adding to said reaction medium a DNA comprising said promoter, such that said DNA-directed RNA polymerase transcribes said DNA, thereby synthesizing said single-stranded RNA.

10. A process according to claim 1, wherein said second oligonucleotide primer further comprises an antisense sequence of a transcription initiation site for said DNA-directed RNA polymerase, said antisense sequence of said transcription initiation site being operatively linked to said antisense sequence of said promoter.

11. A process according to claim 1, wherein said RNA-directed DNA polymerase is a retrovirus reverse transcriptase.

12. A process according to claim 1, wherein said DNA-directed DNA polymerase lacks exonuclease activity.

13. A process according to claim 1, wherein all DNA polymerases in said reaction medium lack exonuclease and DNA endonuclease activity.

14. A process according to claim 1, wherein said DNA-directed DNA polymerase is DNA polymerase α or DNA polymeraseβ.

15. A process for the detection of amplified products comprising the steps of :

(a) amplifying a sample nucleic acid under conditions to generate amplified product;

(b) mixing said amplified product with two binding species comprising

(i) an ECL labeled binding species which interacts with a trimolecular complex with the amplified nucleic acid and bivalent binding species;

(ii) a bivalent binding species which interacts with a trimolecular complex with the amplified nucleic acid and ECL labeled binding species;

to form a binding complex reaction;

(c) incubating said binding complex reaction under conditions which allow the formation of a trimolecular complex of amplified product, ECL labeled binding species, and bivalent binding species;

(d) capturing said trimolecular complex via the bivalent binding species' remaining binding site to a solid phase; and

(e) quantitating ECL label captured on the solid phase.

16. A process according to claim 15 wherein said amplification conditions are isothermal.

17. A process according to claim 15 wherein said binding species is selected from the group consisting of an antibody:antigen, oligonucleotide:oligonucleotide, oligonucleotide:antibody, oligonucleotide:antigen, DNA:DNA, DNA:RNA, RNA:RNA, DNA:RNA:DNA, Biotin-DNA:DNA-ECL labeled, receptor:ligand, and DNA binding protein.

18. A process for the quantitative measurement of a sample comprising the steps of:

(a) amplifying an unknown sample with a known sample by the same primers, said known sample containing a non-homologous sequence to a sequence of said unknown sample, to

form a mixture of amplified product containing copies of the unknown sample and the known sample;

(b) taking said mixture of amplified product and separately quantitating the unknown sample and the known sample comprising the steps of:

(i) separately mixing the mixture of amplified product with two binding species, one specific for each of the known sample sequence and unknown sample sequence containing:

(1) an ECL labeled binding species which interacts with a trimolecular complex with the amplified nucleic acid and bivalent binding species;
(2) a bivalent binding species which interacts with a trimolecular complex with the amplified nucleic acid and ECL labeled binding species;

to form a binding complex reaction;

(c) incubating said binding complex reaction under conditions which allow the formation of a trimolecular complex of amplified product, ECL labeled binding species, and bivalent binding species;

(d) capturing said trimolecular complex via the bivalent binding species' remaining binding site to a solid phase; and

(e) quantitating ECL for said known sample and said unknown sample and then determine the amount of unknown sample in the unamplified starting reaction.

19. A process according to claim 18 wherein said amplification is isothermal.

20. A process according to claim 18 wherein said sample is selected from the group consisting of nucleic acids, amplified products, and synthetic DNA.